

**Microbial iron reduction in a sub-glacial environment: A potential strategy for microbial life on other icy worlds**

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**Introduction**

Ferric iron is an abundant potential electron acceptor in many cold environments on Earth<sup>1</sup> and on other icy worlds in the solar system, such as Mars<sup>2</sup>. The abundance of this potential energy source has led to the suggestion that dissimilatory iron reduction may have been one of the earliest forms of microbial respiration<sup>3</sup>. Moreover, evidence for pervasive past glaciation on both Earth<sup>4</sup> and Mars<sup>5</sup> suggests that large subglacial habitats containing abundant ferric iron have existed on both planets. Such habitats may have provided a suitable set of conditions for the emergence of dissimilatory iron reduction as an energetic strategy for cold tolerant microbes.

Sparse data exists on dissimilatory iron reducers in subglacial environments on Earth, and definitive evidence linking such organisms to the active, in situ reduction of ferric iron in these habitats is currently lacking. Obtaining such evidence is key to understanding the physiology and biosignatures of dissimilatory iron reducing microorganisms in subglacial environments. The presence of hematite and heterotrophic hematite-reducing microorganisms, along with significant organic carbon in the subglacial sediment of Robertson Glacier (RG), in the Canadian Rocky Mountains, make this glacier an ideal system in which to study subglacial iron-reducers. The objectives of the research supported by this field expedition are to: *1. Demonstrate the presence of dissimilatory iron reducing microorganisms that are actively respiring on ferric iron in the subglacial environment of RG, and 2. Characterize the ability of RG iron reducer(s) to respire on various carbon and ferric mineral sources, and establish rates of ferrous iron reduction for each combination of carbon source and ferric mineral.*

Successful completion of these objectives will provide the first definitive evidence of active, in-situ microbial iron reduction in a subglacial environment. Physiological and phylogenetic data obtained from this study will provide insight into the importance of dissimilatory iron reduction in cold environments and its potential role in the emergence of life in the solar system. Characterizing the iron reduction capabilities of microbial dissimilatory iron reducers in cold environments on Earth will assist in determining the conditions necessary to support iron reducing microorganisms which could potentially exist in cold extraterrestrial environments, such as Mars and other icy worlds.

**Field Site**

Robertson Glacier (RG) is located in Kanaskis country, Alberta, Canada (115°20'W, 50°44'N) adjacent to the Northern flank of the Haig Icefield (Fig 1). The hydrology and mineralogy of the glacier have been previously described<sup>5</sup>. The glacier ranges in elevation from 2370 m to 2900 m, and is approximately 3 km in length. Normally, two meltwater streams (an Eastern and Western stream) drain from the glacial

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terminus and flow Northward through the flat till plain upon which RG terminates, and down through the glacial valley. The volume and rate of flow of these streams varies significantly with the seasons, being strongest in late summer and diminishing with the decreasing temperatures of late fall and early winter. Meltwater temperature typically ranges from 0.3 °C to 1.3 °C. Glacially-smoothed bedrock surfaces are visible along RG glacial margins.

## Field Objectives

The 2010 RG field team consisted of three researchers from Montana State University's (MSU) Astrobiology Biogeocatalysis Research center (Dr. John Peters and Dr. Eric S. Boyd from the Department of Chemistry and Biochemistry, and graduate student Matthew R. Urschel from the Department of Microbiology), along with four researchers from Arizona State University Department of Chemistry and Biochemistry (Dr. Everett Shock, Dr. Jeff Havig, and two graduate students). Researchers were housed at the Barrier Lake field station, operated by the University of Calgary Biogeoscience institute. The expedition took place on October 13 through October 17, 2010. The objectives of the field expedition were to:

1. Collect pore water and sediment samples for later experiments aimed at determining pore water chemistry, quantifying and characterizing iron reducing microorganisms, and characterizing the phylogenetic diversity of the microbial population in these sediments
2. Measure important environmental variables at each sample collection site (pH, temperature, electrical conductivity, reduction potential) that determine the site's suitability for microbial iron reduction

## Field Methods and Results



**Day 1, 10/14/2010:** The field team arrived at the terminus of Robertson Glacier at approximately 10:30 AM, local time. The ambient temperature at the site was approximately 2 °C and had been much colder over night. Consequently, most glacial sediments were frozen and impossible to sample for the first 2 hours on site.

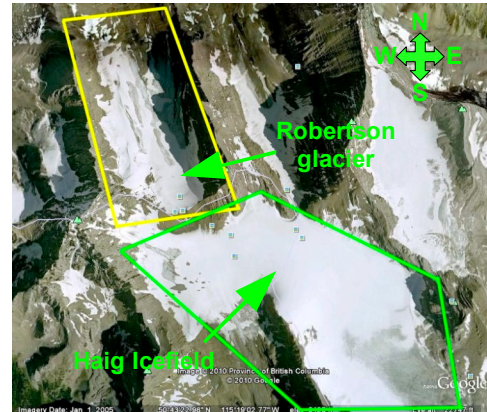


Figure 1. Robertson glacier and Haig Icefield in the Canadian Rocky Mountains.

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**Day 2, 10/16/2010:** Cold weather and heavy snowfall prevented travel to the glacier on 10/15/2010. The weather improved on 10/16/2010, with no snowfall, but colder temperatures continued. Cold weather gear was necessary due to subzero temperatures. The field team arrived at the glacier at approximately 11:00 AM, local time. The ambient temperature was approximately -2 °C, skies were overcast, and windy conditions prevailed. Sediments were frozen, covered with 2-4 inches of snow, and impossible to sample except in close proximity to the melt water stream.

**Objective 1 – Pore water and sediment**

**sampling:** Sampling was possible only in very close proximity to the melt water stream. Water and sediment samples were taken by M. Urschel from a single stream site. Water samples were taken using sterile, 30 ml polyethylene centrifuge tubes. Sediment samples were taken using a flame sterilized garden spade to fill three sterile 50 ml Falcon tubes. Later in the day (at approximately noon, local time) on Day 1, more sunny conditions allowed the sampling of partially-thawed, saturated sediments by M. Urschel at two sites farther from the stream bed. Pits of approximately 15 cm in depth were dug using a flame sterilized garden spade, and allowed to fill with pore water. Water and sediment samples were taken from these sites as described for the stream sites. All samples were flash frozen in a dry-ice/ethanol slurry and stored at -20 °C for transport back to Barrier Lake field station and ultimately to Dr. Mark Skidmore's lab at Montana State University. Sample coupons filled with ferric minerals (hematite or magnetite) and control minerals (calcite or limestone) were placed at the bottom of each pit, to be recovered on a future field expedition and analyzed for the attachment of iron-reducing organisms. Pore water samples will be analyzed by Ion Chromatography (IC) and Total Organic Carbon (TOC) analysis to determine the concentration of ions and organic carbon available to iron reducing microorganisms. DNA will be isolated from sediment samples and analyzed by real time quantitative PCR (qPCR) to determine the abundance of iron reducing microorganisms present in the sediments.





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**Objective 2 - Measure important environmental variables at each sample collection site:** Several environmental variables important to microbial iron reduction were measured in both stream water and pore water at saturated sediment sites. Electrical conductivity (an indicator of the amount of solids dissolved in solution) and temperature were measured using an EC300 handheld conductivity/temperature meter (YSI Inc., Yellow Springs, OH). Oxidation/Reduction potential (ORP, a measure of the solution's tendency to donate electrons to or receive electrons from minerals in solution) was measured using an ORPTestr 10 Waterproof ORP / Redox Tester (Oakton Instruments, Canada). pH was measured using an Orion 290A+ multimeter (ThermoScientific, Beverly, MA). These measurements, along with measurements of dissolved organic carbon and dissolved ions (to be performed at MSU), will be used determine a given site's favorability to microbial iron reduction.



Back to the field station for a well-earned meal!



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